

# New Guidelines for $\delta^{13}\text{C}$ Measurements

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Consistency of  $\delta^{13}\text{C}$  measurements can be improved 39–47% by anchoring the  $\delta^{13}\text{C}$  scale with two isotopic reference materials differing substantially in  $^{13}\text{C}/^{12}\text{C}$ . It is recommended that  $\delta^{13}\text{C}$  values of both organic and inorganic materials be measured and expressed relative to VPDB (Vienna Pee Dee belemnite) on a scale normalized by assigning consensus values of  $-46.6\text{‰}$  to L-SVEC lithium carbonate and  $+1.95\text{‰}$  to NBS 19 calcium carbonate. Uncertainties of other reference material values on this scale are improved by factors up to two or more, and the values of some have been notably shifted: the  $\delta^{13}\text{C}$  of NBS 22 oil is  $-30.03\text{‰}$ .

Differences in measured isotope amount ratios of stable carbon isotopes ( $^{13}\text{C}/^{12}\text{C}$ ), commonly called  $\delta^{13}\text{C}$  values, are used in oceanography, atmospheric sciences, biology, paleoclimatology, geology, environmental sciences, food and drug authentication, and forensic applications. Progress in these fields requires smaller measurement uncertainties to be achieved. Advances require more accurate and intercomparable measurements; for example, a difference of  $0.01\text{‰}$  in  $\delta^{13}\text{C}$  of global atmospheric  $\text{CO}_2$  can equate to a global carbon balance source/sink discrepancy of  $\sim 400\text{ Tg}$  among international monitoring networks.<sup>1</sup> Although advances in instrumentation enable increasingly precise measurements, laboratories measuring the same specimen often disagree by 10 times their reported “uncertainty” of measurement,<sup>2,3</sup> and agreement has not improved appreciably in the last two decades, except in special cases.<sup>4</sup>

Recognizing that two-point calibrations of the  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  scales substantially improved the agreement among laboratories,<sup>5</sup> the International Atomic Energy Agency (IAEA) convened a consultants meeting in 2004 to review stable carbon isotopic reference materials and to recommend a second reference material for two-point normalization of the  $\delta^{13}\text{C}$  scale. Four laboratories (Centrum voor Isotopen Onderzoek, Groningen, Netherlands; Max-Planck-Institute for Biogeochemistry, Jena, Germany; UFZ Leipzig-Halle, Leipzig, Germany; U.S. Geological Survey, Reston, Virginia) performed analytical measurements. Participants at the U.S. National Institute of Science and Technology (NIST) headed the task to estimate consensus means and uncertainties.

## EXPERIMENTAL METHODS

**Mass Spectrometry.** In this study, 1055 state-of-the-art continuous flow elemental-analyzer (EA) mass spectrometry measurements using the general method of Qi et al.<sup>7</sup> were performed on selected organic and inorganic carbon isotopic reference materials. NBS 19 calcium carbonate was adopted for anchoring at high  $^{13}\text{C}$  amount and was assigned the value  $+1.95\text{‰}$  relative to VPDB following recommendations of the IAEA and the International Union of Pure and Applied Chemistry (IUPAC).<sup>8,9</sup>

### Estimating Consensus Means Using the Bayesian Method.

In prior intercomparison exercises, consensus—mean estimation was done for each reference material independently using a statistical model that assumes that, for a reference material  $i$  and laboratory  $j$ , the mean value of the measurement can be decomposed into two components, one specific to the reference material

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( $\mu_i$ ) and one specific to the laboratory ( $\alpha_j$ ). Methods such as those described by Rukhin and Vangel<sup>10</sup> were then used to estimate the consensus mean,  $\mu_i$ , and compute the type A uncertainty<sup>11</sup> associated with it. The  $\alpha_j$  are called *random effects*, which are given a probability distribution with mean 0 and some variance,  $\sigma_L$ , and they are used to represent added variability in the data due to random differences between the laboratories. The type A uncertainty contains a component associated with the estimation of  $\mu_i$  and a component that is an estimate of  $\sigma_L$ .

In this study, advantage has been taken of the fact that in all of the data sets, each laboratory has provided measurements on multiple reference materials. Such data makes it possible to deconvolute the mean value of a measurement into the component  $\mu_i$  and a component  $\alpha_{ij}$ . The  $\alpha_{ij}$  values are given a distribution with mean  $\lambda_j$  and variance  $\tau_j$ . Now  $\lambda_j$  represents a systematic laboratory effect, one present across all reference materials. Larger  $\lambda_j$  values represent larger departures of laboratory  $j$  from the consensus, whereas smaller  $\lambda_j$  values signify more “universal” departures that occur across most of the reference materials (i.e.,  $\alpha_{ij}$  values would be similar across materials). The  $\mu_i$  are again estimated to provide the consensus mean for each reference material. These estimates are more accurate than the “one reference material at a time” estimates because they have been adjusted for systematic laboratory effects. Furthermore, the type A uncertainty associated with these consensus means is smaller than that of the corresponding “one reference material at a time” estimates. In this study, the estimation of the  $\mu_i$  and their uncertainties—the  $\lambda_j$  and the  $\tau_j$  values—was done using WinBUGS,<sup>12,13</sup> a program using Markov Chain Monte Carlo computation with a Bayesian hierarchical model having noninformative priors on all of the hyperparameters.<sup>14</sup>

**Evaluation of Uncertainty.** The strategy for evaluating standard uncertainty was guided by ISO-GUM.<sup>11</sup> The multivariate Bayesian determination of consensus means (described above) calculates standard errors about those means. These standard errors are fairly consistent across all materials and compositions, and they may be considered to represent type A standard uncertainties ( $u_A$ ). For reference materials with  $\delta^{13}\text{C}$  values close to +1.95‰ (the realization point of the VPDB scale), the type A uncertainty is a good estimate of the total uncertainty because scale effects approach zero. However, it is well-known that measurement results become less reproducible as the  $\delta^{13}\text{C}$  difference between the sample and the VPDB realization point increases, so although the normalization procedure described here substantially improves measurement reproducibility, analytical limitations still impose scale discrepancies. These discrepancies may be considered the result of type B uncertainty, which is

observed in the normalized data (Tables S-1 and S-2). A general and simple model for type B uncertainty was extracted from the normalized data (eq 1), where the proportionality constant  $k$  was estimated to be 0.001.

$$u_B = |0.00195 - \delta^{13}\text{C}| \cdot k \quad (1)$$

Combined standard uncertainties ( $u_C$ ) were then determined through eq 2.

$$u_C = \sqrt{u_A^2 + u_B^2} \quad (2)$$

An expanded uncertainty ( $U = 2u_C$ ) about the recommended value provides an interval that has about a 95% probability of encompassing the true value.

## RESULTS

The measurements of  $\delta^{13}\text{C}$  values are shown in Tables S-2 and S-3. To normalize  $\delta^{13}\text{C}$  measurements, L-SVEC lithium carbonate (NIST RM 8545) was selected as the low- $^{13}\text{C}$ -content scale anchor because EA  $\delta^{13}\text{C}$  values of amounts as small as 0.3 mg are statistically identical, and carbonates are easily prepared for analysis using  $\text{H}_3\text{PO}_4$ . L-SVEC was assigned a  $\delta^{13}\text{C}$  consensus value of −46.6‰ on the basis of high-accuracy dual-inlet mass spectrometry measurements (optimized to minimize memory and isotopic fractionation) by Ghosh et al.,<sup>15</sup> who determined a value of  $-46.607 \pm 0.057\text{‰}$  ( $u_C$ ), and by Verkouteren and Klinedinst,<sup>4</sup> who calculated a dual-inlet value of  $-46.57 \pm 0.13\text{‰}$  ( $u_C$ ) that was based on the relationship between  $\delta^{17}\text{O}$  and  $\delta^{18}\text{O}$  of 0.528<sup>16</sup> and an  $^{17}\text{O}/^{16}\text{O}$  ratio in VSMOW reference water of 0.000 386 913.<sup>17</sup> Recommended  $\delta^{13}\text{C}$  values (Table 1) were determined by multivariate Bayesian analysis. Recommended  $\delta^{13}\text{C}$  values also were determined for three  $\text{CO}_2$  gases (NIST RM 8562, RM 8563, and RM 8564) and three  $\text{CaCO}_3$  reference materials (IAEA-CO-1, IAEA-CO-8, and NBS 18) because high-quality data were available.<sup>4,15</sup>

## DISCUSSION

Improvement in data consistency was determined by comparing the variations in newly normalized data to unnormalized data in several studies<sup>3,4</sup> for all reference materials with values more negative than −25‰. The average variations (standard deviations) in results across laboratories were lowered 39–47%. As a result, uncertainties in value assignments were also improved, for which combined standard uncertainties were lowered by factors of 2–3 for specific materials (Figure 1).

In August 2005 at IUPAC’s 43rd General Assembly in Beijing, the Commission on Isotopic Abundances and Atomic Weights accepted the recommendations of this IAEA panel that  $\delta^{13}\text{C}$  values of all carbon-bearing materials be measured and expressed relative

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**Table 1. Reference  $\delta^{13}\text{C}$  Values of Stable Carbon Isotopic Reference Materials<sup>a,b</sup>**

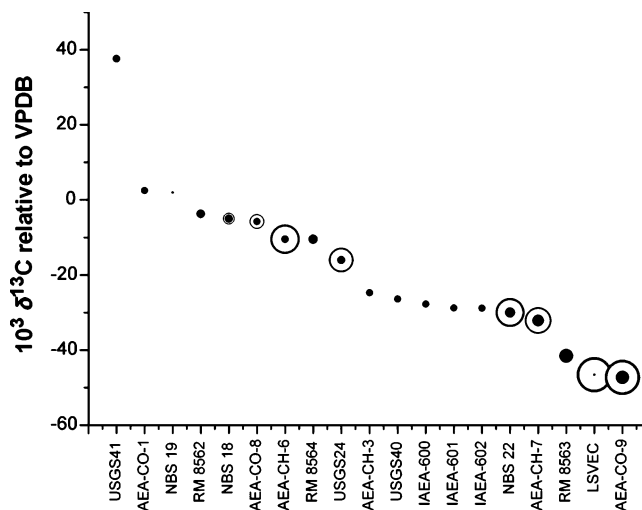
name	description	$\delta^{13}\text{C} \times 10^3$
USGS41	L-glutamic acid	+37.63 <sup>c</sup>
IAEA-CO-1	calcium carbonate	+2.49 <sup>d</sup>
NBS 19	calcium carbonate	+1.95
RM 8562	carbon dioxide	-3.72
NBS 18	calcium carbonate	-5.01 <sup>e</sup>
IAEA-CO-8	calcium carbonate	-5.76 <sup>f</sup>
IAEA-CH-6	sucrose	-10.45
RM 8564	carbon dioxide	-10.45
USGS24	graphite	-16.05 <sup>g</sup>
IAEA-CH-3	cellulose	-24.72
USGS40	L-glutamic acid	-26.39 <sup>h</sup>
IAEA-600	caffeine	-27.77
IAEA-601	benzoic acid	-28.81
IAEA-602 <sup>i</sup>	benzoic acid	-28.85
NBS 22	oil	-30.03 <sup>k</sup>
IAEA-CH-7	polyethylene	-32.15
RM 8563	carbon dioxide	-41.59 <sup>j</sup>
L-SVEC	lithium carbonate	-46.6
IAEA-CO-9	barium carbonate	-47.32 <sup>m</sup>

<sup>a</sup> Recommendations from a consultants meeting of the International Atomic Energy Agency. Analytical data in this study are shown in Tables S-2 and S-3, and estimates of uncertainty are provided in Table S-4 of the Supporting Information. <sup>b</sup>  $\delta^{13}\text{C}$  values expressed relative to VPDB ( $\delta^{13}\text{C}$  of NBS19  $\equiv +1.95\text{‰}$ ) and normalized to  $\delta^{13}\text{C}$  of L-SVEC  $\equiv -46.6\text{‰}$ . <sup>c</sup> Qi et al. value<sup>7</sup> (normalized to  $\delta^{13}\text{C}$  of L-SVEC  $\equiv -46.6\text{‰}$ ) of +37.77 $\text{‰}$  ( $u_c = 0.09\text{‰}$ ) is within 2  $u_c$  of recommended value (Table S-5). <sup>d</sup> Qi et al. value<sup>7</sup> (normalized to  $\delta^{13}\text{C}$  of L-SVEC  $\equiv -46.6\text{‰}$ ) of +2.48 $\text{‰}$  ( $u_c = 0.06\text{‰}$ ), Stichler Bayesian value<sup>3</sup> (normalized to  $\delta^{13}\text{C}$  of L-SVEC  $\equiv -46.6\text{‰}$ ) of +2.46 $\text{‰}$  ( $u_c = 0.03\text{‰}$ ), and Ghosh et al. value<sup>15</sup> (normalized to  $\delta^{13}\text{C}$  of L-SVEC  $\equiv -46.6\text{‰}$ ) of +2.49 $\text{‰}$  ( $u_c = 0.03\text{‰}$ ) are all within 1  $u_c$  of recommended value (Tables S-5–7). <sup>e</sup> Stichler Bayesian value<sup>3</sup> (normalized to  $\delta^{13}\text{C}$  of L-SVEC  $\equiv -46.6\text{‰}$ ) of -5.78 $\text{‰}$  ( $u_c = 0.03\text{‰}$ ) and Ghosh et al. value<sup>15</sup> (normalized to  $\delta^{13}\text{C}$  of L-SVEC  $\equiv -46.6\text{‰}$ ) of -5.76 $\text{‰}$  ( $u_c = 0.03\text{‰}$ ) are within 1  $u_c$  of recommended value (Tables S-6 and S-7). <sup>f</sup> Stichler Bayesian value<sup>3</sup> (normalized to  $\delta^{13}\text{C}$  of L-SVEC  $\equiv -46.6\text{‰}$ ) of -16.06 $\text{‰}$  ( $u_c = 0.05\text{‰}$ ) is within 1  $u_c$  of recommended value (Table S-6). <sup>g</sup> Qi et al. value<sup>7</sup> (normalized to  $\delta^{13}\text{C}$  of L-SVEC  $\equiv -46.6\text{‰}$ ) of -26.31 $\text{‰}$  ( $u_c = 0.06\text{‰}$ ) is within 2  $u_c$  of recommended value (Table S-5). <sup>h</sup> IAEA-602 is listed here for information purposes only. It is not recommended for routine carbon isotopic calibration because it is enriched in  $^{17}\text{O}$  and  $^{18}\text{O}$ , and its  $^{17}\text{O}/^{18}\text{O}$  ratio no longer reflects that of natural terrestrial materials. <sup>i</sup> Qi et al. value<sup>7</sup> (normalized to  $\delta^{13}\text{C}$  of L-SVEC  $\equiv -46.6\text{‰}$ ) of -29.99  $\pm$  0.05 $\text{‰}$  is within 1  $u_c$  of recommended value (Table S-5) and is in accord with Stalker et al.<sup>19</sup> observation that prior  $\delta^{13}\text{C}$  values of NBS 22 and other organic reference materials are more positive by  $\sim 0.25\text{‰}$ . <sup>j</sup> Ghosh et al. value<sup>15</sup> (normalized to  $\delta^{13}\text{C}$  of L-SVEC  $\equiv -46.6\text{‰}$ ) of -41.61 $\text{‰}$  ( $u_c = 0.05\text{‰}$ ) is within 1  $u_c$  of recommended value (Table S-7). <sup>k</sup> Both Stichler Bayesian value<sup>3</sup> (normalized to  $\delta^{13}\text{C}$  of L-SVEC  $\equiv -46.6\text{‰}$ ) of -47.31 $\text{‰}$  ( $u_c = 0.06\text{‰}$ ) and Verkouteren and Klinedinst [Table 10-D] Bayesian value<sup>4</sup> of -47.38 $\text{‰}$  ( $u_c = 0.06\text{‰}$ ) are within 1  $u_c$  of recommended value (Tables S-6 and S-8).

to VPDB on a scale normalized by assigning consensus values of -46.6 $\text{‰}$  to L-SVEC lithium carbonate and +1.95 $\text{‰}$  to NBS 19 calcium carbonate, and authors should clearly state so in their reports. Authors are encouraged to report their measurement results for  $\delta^{13}\text{C}$  values of NBS 22 oil, USGS41 L-glutamic acid, IAEA-CH-6 sucrose, or other internationally distributed reference materials, as appropriate for the measurement method concerned.

## CONCLUSIONS

Anchoring the  $\delta^{13}\text{C}$  scale at a second point by assigning a consensus value of -46.6 $\text{‰}$  to the reference material L-SVEC will



**Figure 1.** Improvement in combined standard uncertainty for  $\delta^{13}\text{C}$  reference materials compared with previously assessed uncertainty. Sizes of solid points indicate newly estimated uncertainties (largest solid circle is equivalent to an uncertainty of 0.06 $\text{‰}$ ), diameter of open circles (older materials only) indicate their previously estimated uncertainties (largest open circle is equivalent to an uncertainty of 0.15 $\text{‰}$ ). By consensus,  $\delta^{13}\text{C}$  values of NBS 19 and L-SVEC have no associated uncertainty on the normalized scale.

help establish a worldwide equivalence among laboratories, that is, the ability of independent laboratories to measure and to report intercomparable  $\delta^{13}\text{C}$  values that, for common samples, agree with one another within measurement uncertainty. With these guidelines, the  $\delta^{13}\text{C}$  scale can now be anchored at two points, as has been done for the  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  scales during the last 3 decades, to improve data reliability of a wide variety of programs, including authenticating the origin of pharmaceuticals to combat counterfeiting, addressing megaton discrepancies in global atmospheric carbon inventories, and harmonizing results from international laboratories that test for use of anabolic steroids at the Olympic Games.

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## SUPPORTING INFORMATION AVAILABLE

Excel file having eight worksheets containing Tables S-1–8 as noted in text. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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